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colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human CEACAM5 in serum and plasma.

general information

Catalogue Number	KE00042
Product Name	CEACAM5 ELISA Kit
Species cross-reactivity	Human CEACAM5
Range (calibration Range)	125 - 8000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**1048** (Human)
SwissProt	**P06731** (Human)

kit components & storage

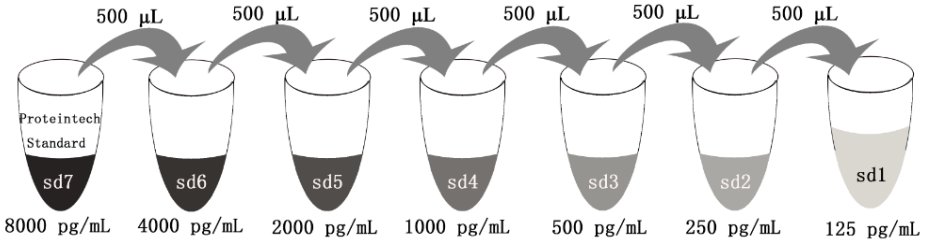
Microplate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Store at -20°C for six months
Standard - 16000 pg/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-ag - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	3 pieces	

NB: Do not use the kit after the expiration date.

Sample Diluent PT 1-ag is for Standard and Samples.

Detection Diluent is for Detection antibody and HRP-conjugated antibody.

*Add 2 mL Sample Diluent PT 1-ag in Standard, This reconstitution gives a stock solution of 8000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-ag	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00042 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The CEACAM5 ELISA kit is to be used to detect and quantify protein levels of endogenous CEACAM5. The assay recognizes human CEACAM5. A polyclonal antibody specific for CEACAM5 has been pre-coated onto the microwells. The CEACAM5 protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody specific for CEACAM5 is added to detect the captured CEACAM5 protein. For signal development, horseradish peroxidase (HRP)-conjugated antibody is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

background

CEACAM5, also known as carcinoembryonic antigen (CEA) or CD66e, is a member of the immunoglobulin superfamily, mainly serving as a cell adhesion molecule mediating intercellular contact by both homophilic and heterophilic binding. CEACAM5 is overexpressed in a wide variety of human cancers, including colon, breast, and lung. It inhibits anoikis and plays a role in tumorigenesis and metastasis. CEACAM5 is not usually present in the blood of healthy adults. However, it has been found that serum from people with colorectal, gastric, pancreatic, lung, and breast carcinoma has higher levels of CEACAM5 than healthy people. CEACAM5 is a tumor marker and is routinely exploited for diagnosis.

sample preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

safety notes

This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

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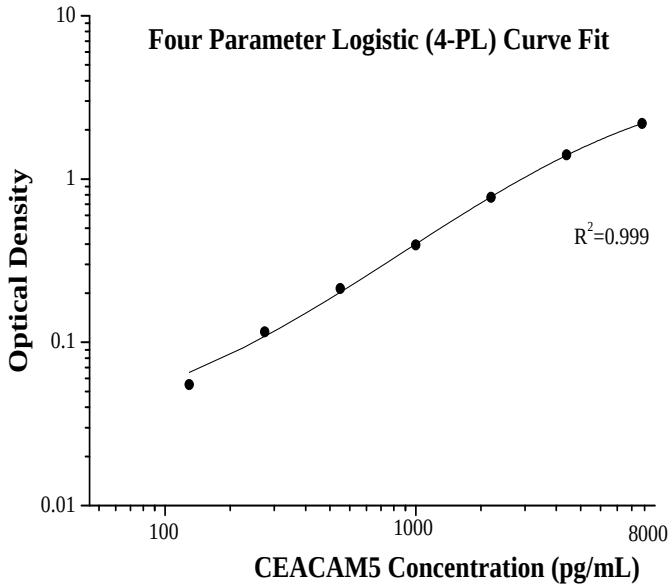
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	**120 min**	4 times	Cover Wells
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells
4	TMB Substrate	100 µL	15-30 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

typical data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.088	0.089	—
	0.09		
125	0.139	0.144	0.055
	0.149		
250	0.207	0.2045	0.1155
	0.202		
500	0.291	0.3025	0.2135
	0.314		
1000	0.465	0.4835	0.3945
	0.502		
2000	0.87	0.8625	0.7735
	0.855		
4000	1.529	1.492	1.403
	1.455		
8000	2.321	2.2785	2.1895
	2.236		

precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/ml)	4689	1010.2	255.4	5177	1131.6	250
SD	144	34.6	18.6	302.2	51.6	22.8
CV%	3.1	3.4	7.3	5.8	4.6	9.1

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recovery

The recovery of CEACAM5 spiked to three different levels in four samples throughout the range of the assay in human plasma averaged 94%, ranging from 78%-119%.

sensitivity

The minimum detectable dose of human CEACAM5 is 64 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of CEACAM5 in human plasma and diluted with the appropriate **Sample Diluent PT 1-ag** to produce samples with values within the dynamic range of the assay. (The samples were initially diluted 1:1)

		Citrate plasma
1:2	Average% of Expected	77
	Range(%)	76-78
1:4	Average% of Expected	81
	Range(%)	81-82
1:8	Average% of Expected	90
	Range(%)	83-93
1:16	Average% of Expected	96
	Range(%)	87-102

references

1. Duffy MJ, *et al*. Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful? Clin Chem. 47(4):624-30 (2001).
2. Zheng C, *et al*. A novel anti-CEACAM5 monoclonal antibody, CC4, suppresses colorectal tumor growth and enhances NK cells-mediated tumor immunity. PLoS One. 6(6):e21146 (2011).
3. Ordonez C, *et al*. GPI-anchored CEA family glycoproteins CEA and CEACAM6 mediate their biological effects through enhanced integrin alpha5beta1-fibronectin interaction. J Cell Physiol. 210(3):757-65 (2007).
4. Lee H, *et al*. Diagnostic significance of serum HMGB1 in colorectal carcinomas. PLoS One. 7(4):e34318 (2012).